#2

Primary lymphedema (clinical classification).

Diagnosis	Frequency[22,31,32,39] (% of all primary forms)
Congenital (onset <2 years after birth)	6-12
Familial, autosomal dominant (Nonne-Milroy disease)	
Familial, non-dominant inheritance	
Sporadic (most common congenital form)	
Lymphedema precox (onset between 2-35 years)	77-94
Familial, autosomal recessive (Meige disease)	
Sporadic (83-94% of all lymphedema precox)	
Lymphedema tarda (onset after 35 years of age)	11

FIG. 1A

Functional Classification of Primary Lymphedema

	Distal Obliteration (80%)	Proximal Obliteration (10%)	Hyperplasia* (10%)
Gender	Female	Male orFemale	Male orFemale
Onset			
Time	Puberty	Any age	Congenital
Location	Ankle; bilateral	Whole leg, thigh; unilateral	Whole leg; unilateral or bilateral
Progression	Slow	Rapid	Progressive
Family history	Frequently positive	None	Frequently positive

FIG. 1B

Secondary lymphedema

Blockade at the level of the lymph node

- Regional lymph node dissection
  - Axillary (post-mastectomy lymphedema)
  - Pelvic and para-aortic (leg and groin lymphedema)
  - Neck (head and neck lymphedema)
- Neoplastic disease
  - Hodgkin lymphoma
  - Metastatic cancer
  - Prostate cancer
  - Cervical cancer
  - Breast cancer
  - Melanoma

Disruption or obliteration of lymphatic channels

- Surgery, e.g. ilio-femoral bypass
- Direct injury, e.g. trauma of the medial aspect of the thigh
- Radiation-induced fibrosis
- Neoplastic infiltration of lymphatic channels
- Rheumatiod arthritis
- Filariasis
- Recurrent infection, e.g. erysipelas

FIG. 1C

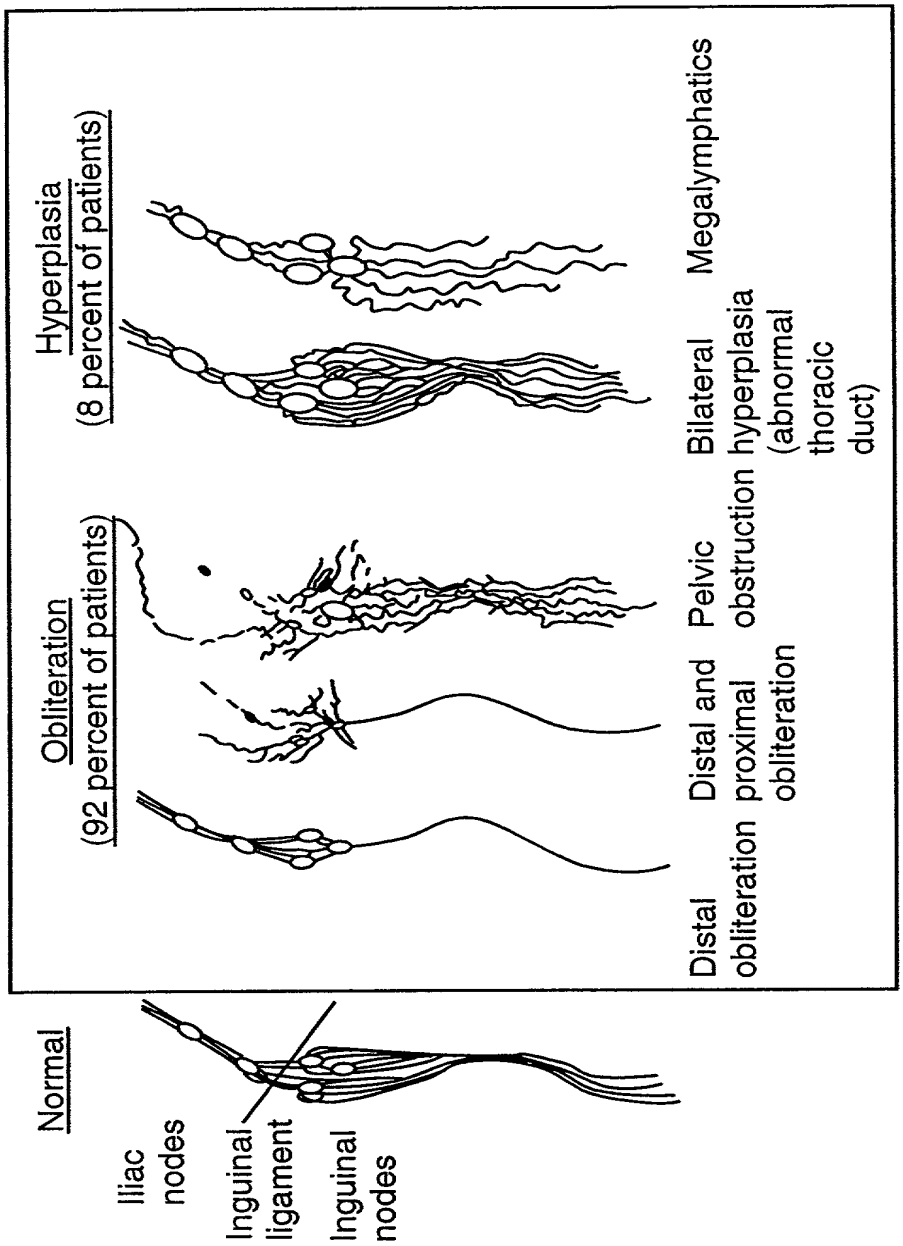

Lymphangiographic Patterns
Normal vs. Primary Lymphedema
Normal
Iliac nodes
Inguinal ligament
Inguinal nodes
Obliteration
(92 percent of patients)
Distal obliteration
Distal and proximal obliteration
Pelvic obstruction
Hyperplasia
(8 percent of patients)
Bilateral hyperplasia (abnormal thoracic duct)
Megalymphatics

FIG. 2

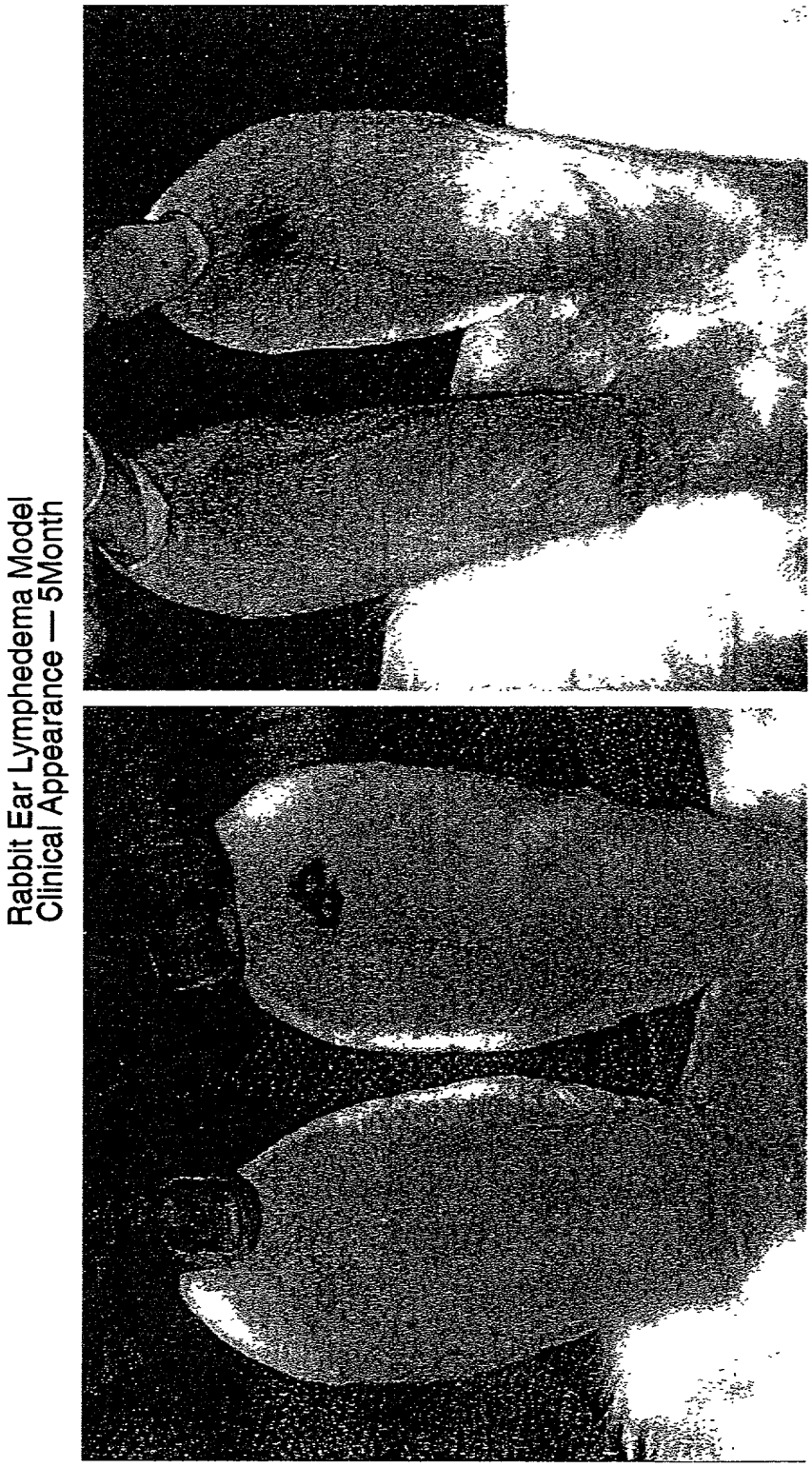

Rabbit Ear Lymphedema Model
Clinical Appearance — 5Month
VEGF-2
Control

FIG. 3

Rabbit Ear Lymphedema Model
Lymphoscintigraphy — 5 Month Post-Op

VEGF-2

Control

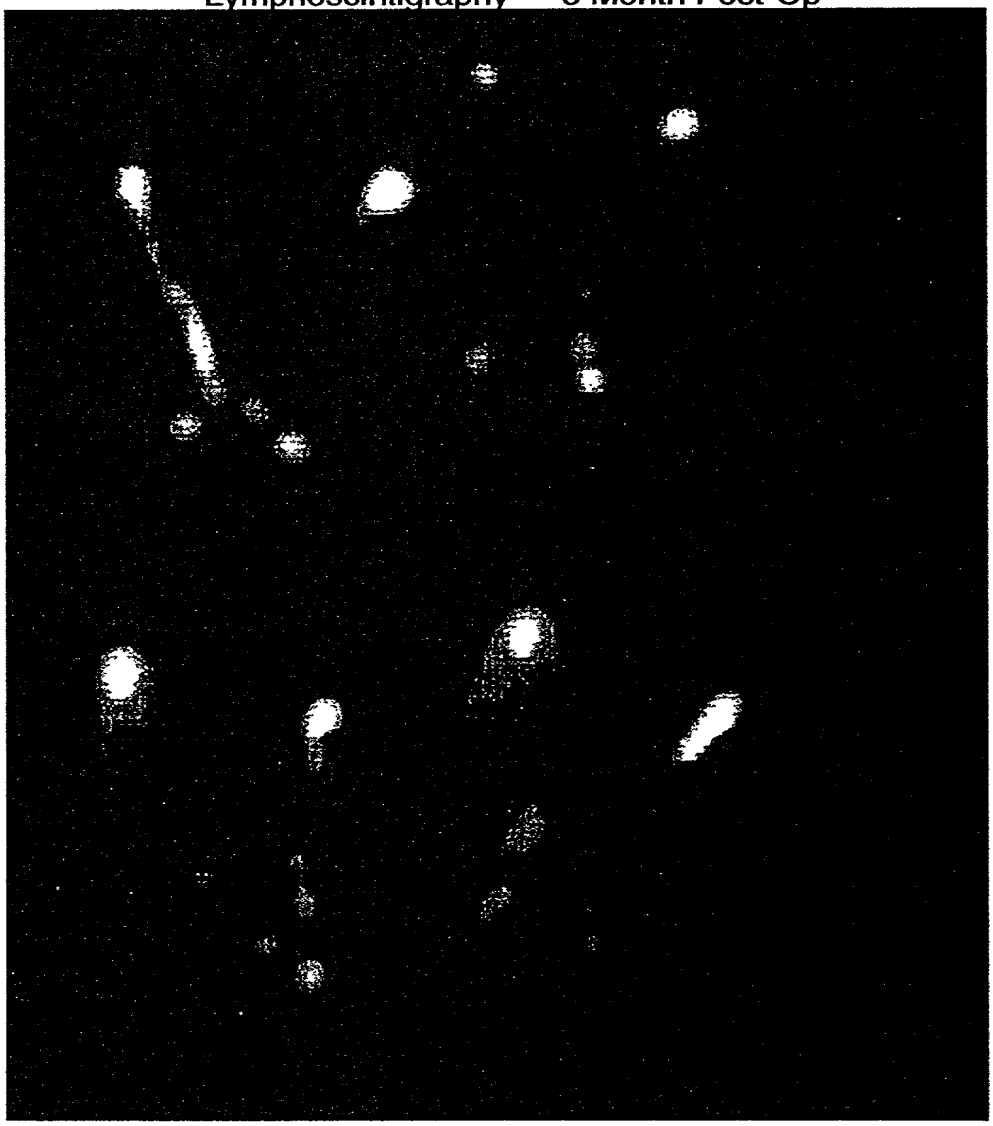
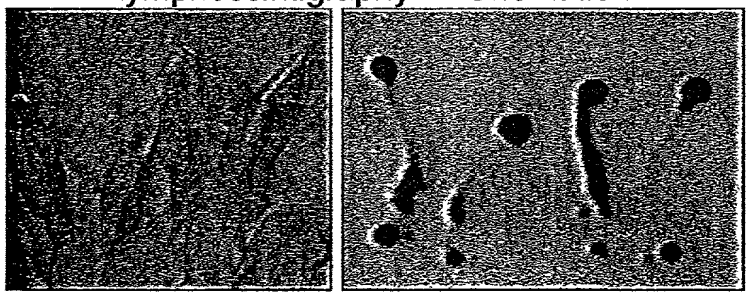


FIG. 4

Rabbit Ear Lymphedema Model
Lymphoscintigraphy — Orientation



Right Operated

Left Normal

FIG. 5

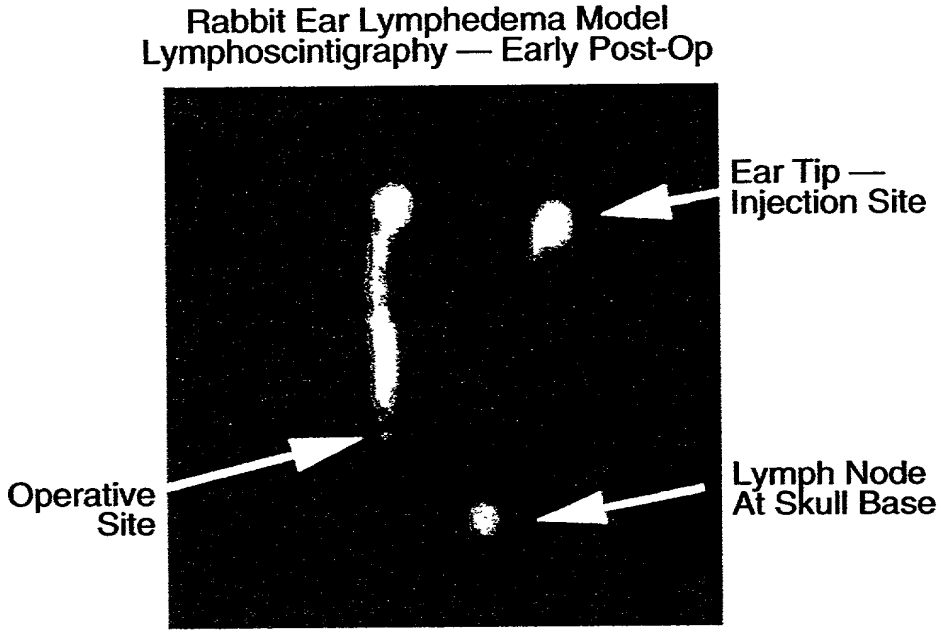

Rabbit Ear Lymphedema Model
Lymphoscintigraphy — Early Post-Op
Ear Tip —
Injection Site
Operative
Site
Lymph Node
At Skull Base

FIG. 6

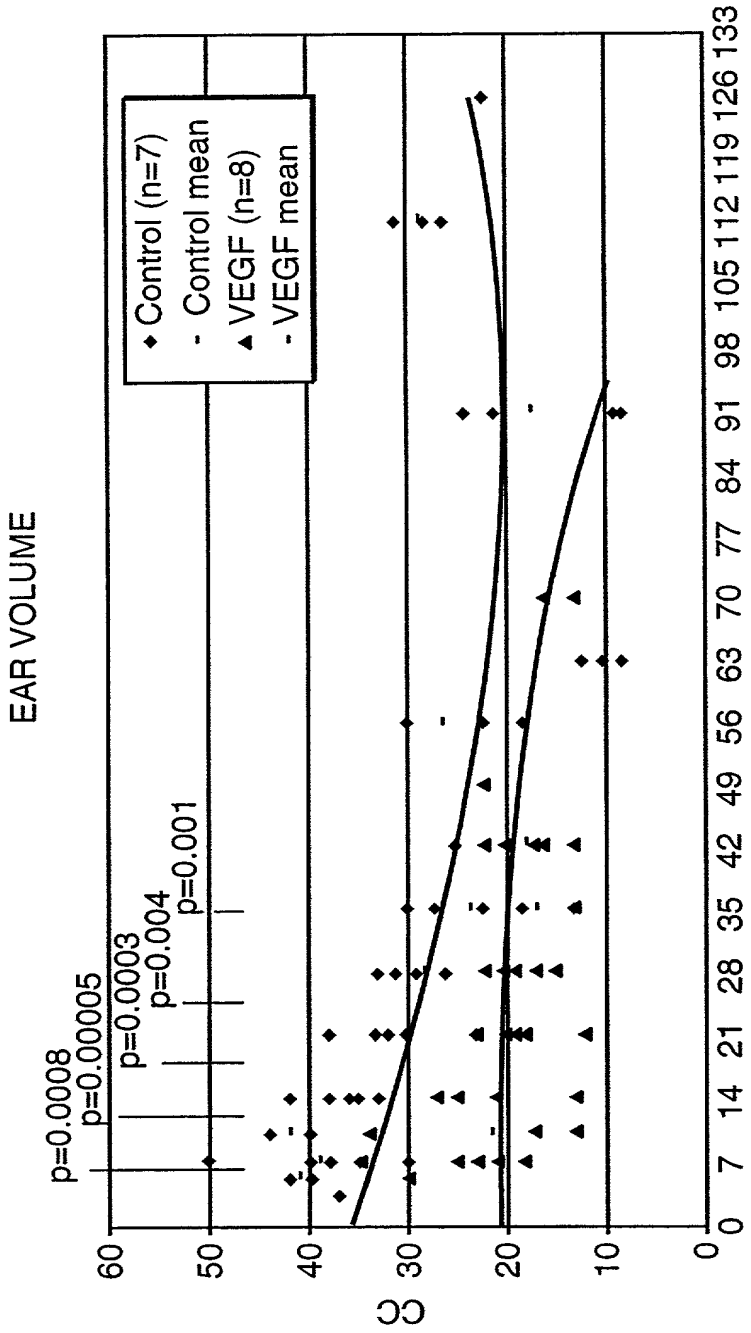

EAR VOLUME
cc
DAY
Control (n=7)
Control mean
VEGF (n=8)
VEGF mean
p=0.0008
p=0.00005
p=0.0003
p=0.004
p=0.001
0
10
20
30
40
50
60
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105 112 119 126 133

FIG. 7

Rabbit Ear Lymphedema Model
3 Days Post-Op

FIG. 8

Human Lymphoscintigraphy
Right Lower Extremity

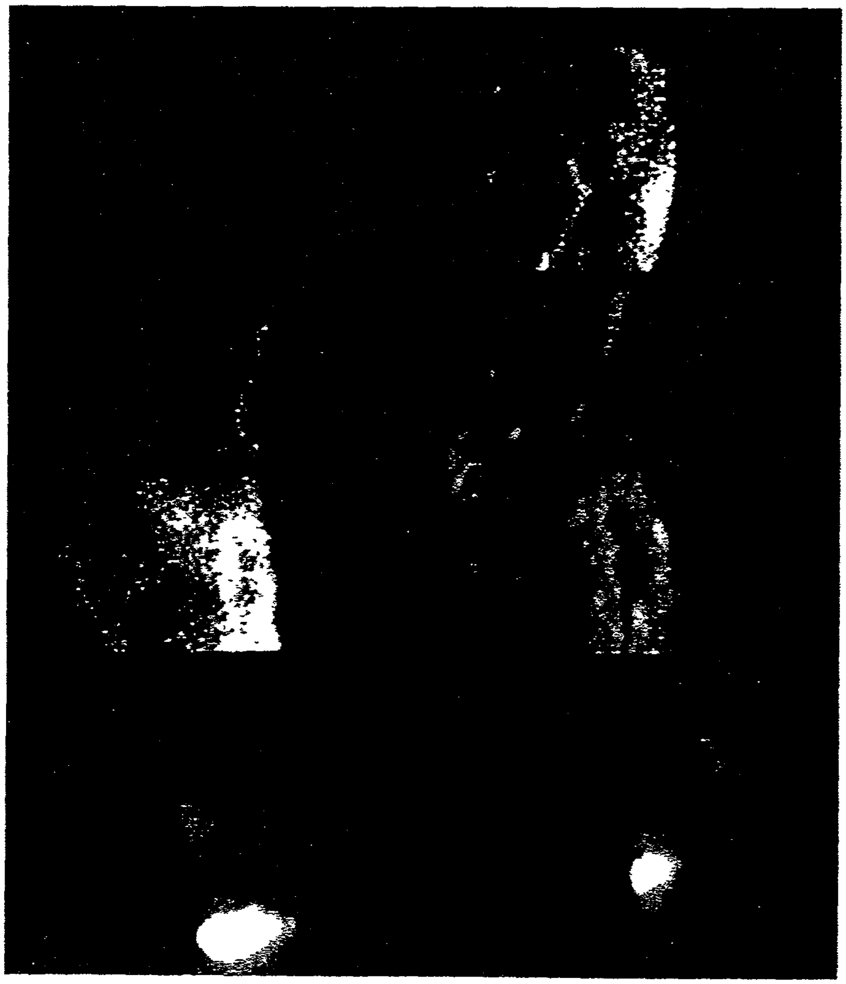


FIG. 9

Ultrasound Imaging of Intra-Muscular
VEGF-2 Gene Transfer: Lymphedema

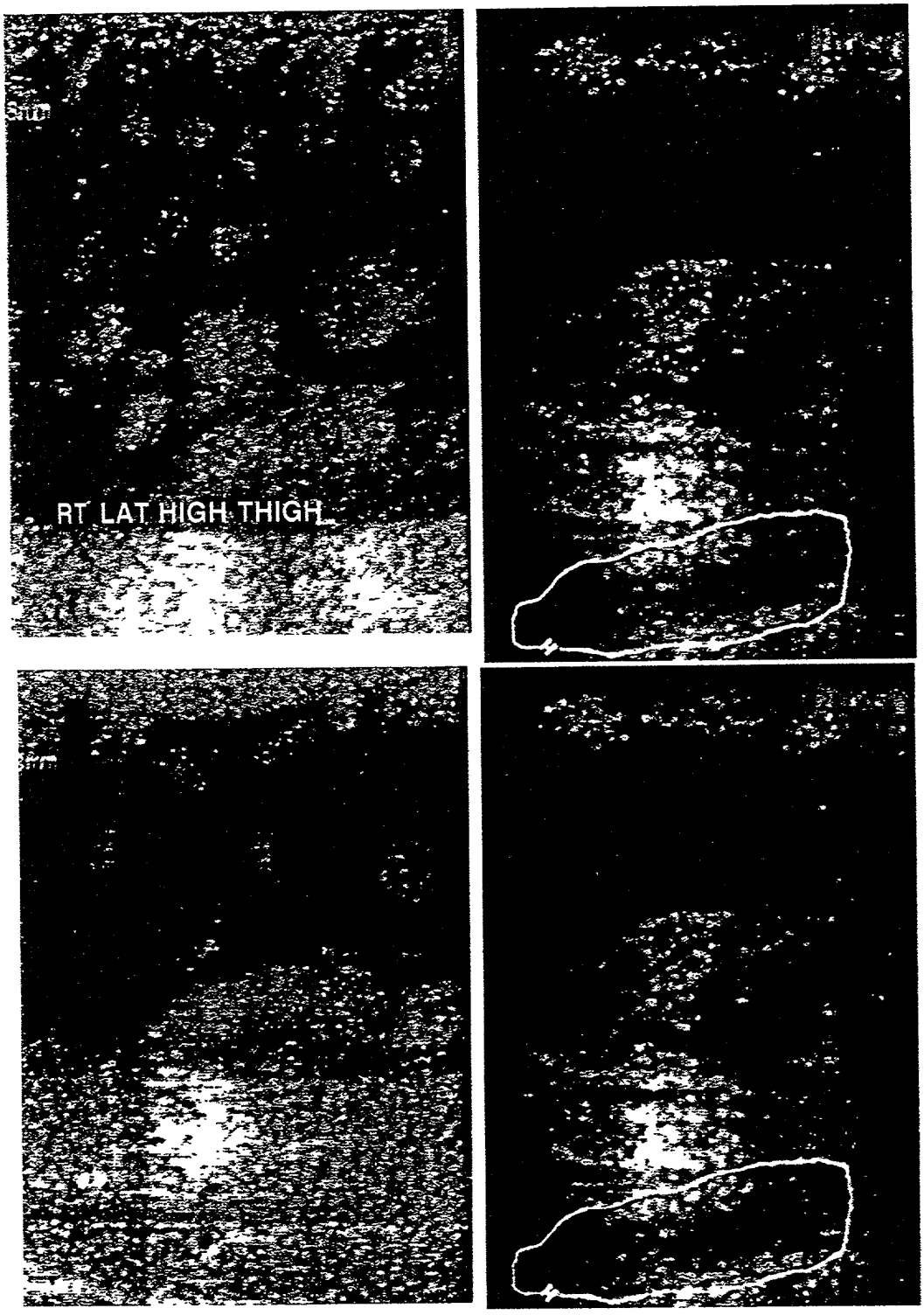


FIG. 10

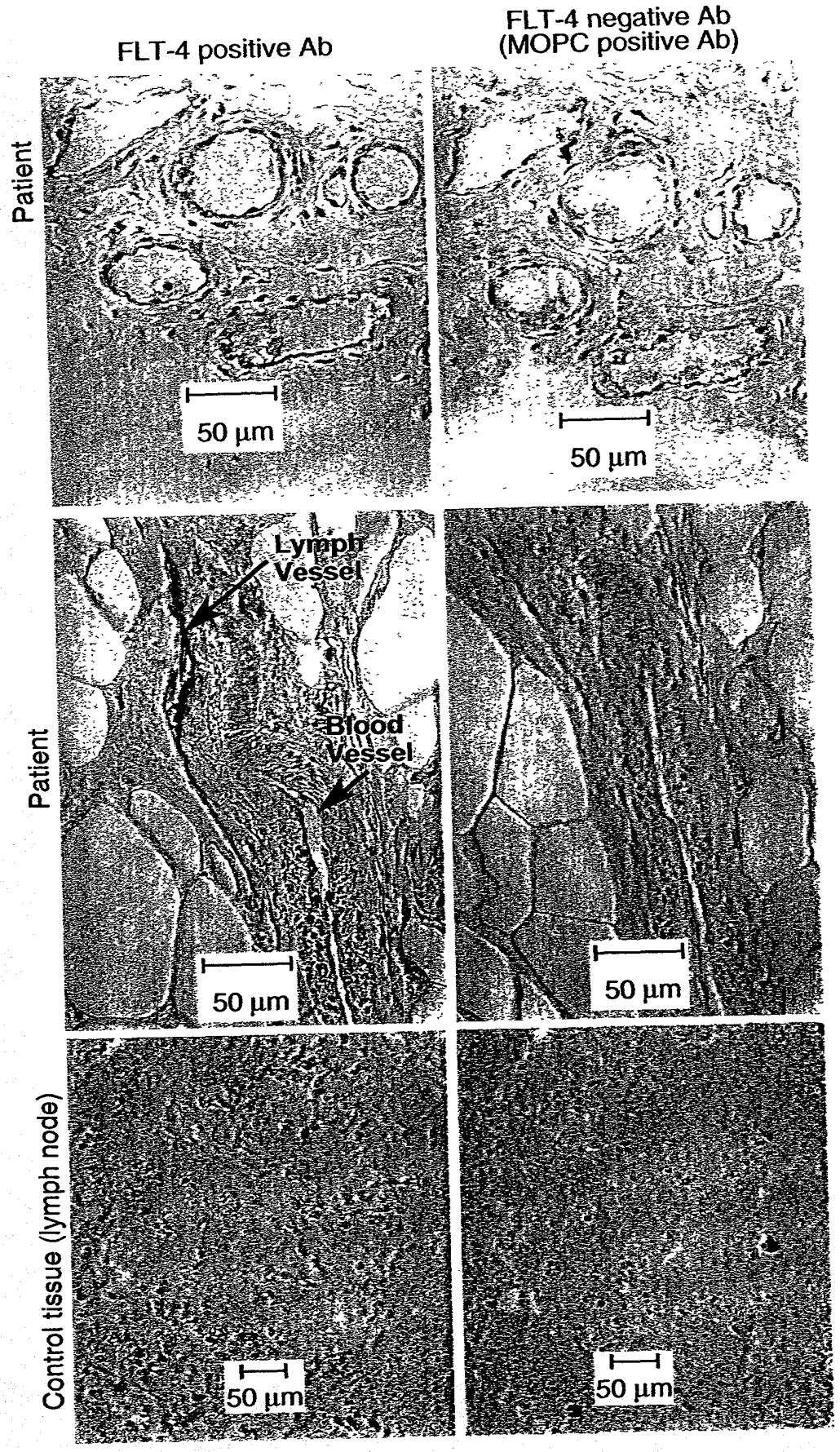

FLT-4 positive Ab
FLT-4 negative Ab
(MOPC positive Ab)
Patient
Patient
Control tissue (lymph node)
Lymph Vessel
Blood Vessel
50 µm
50 µm
50 µm
50 µm
50 µm
50 µm

FIG. 11

FIG. 12B

FIG. 12A

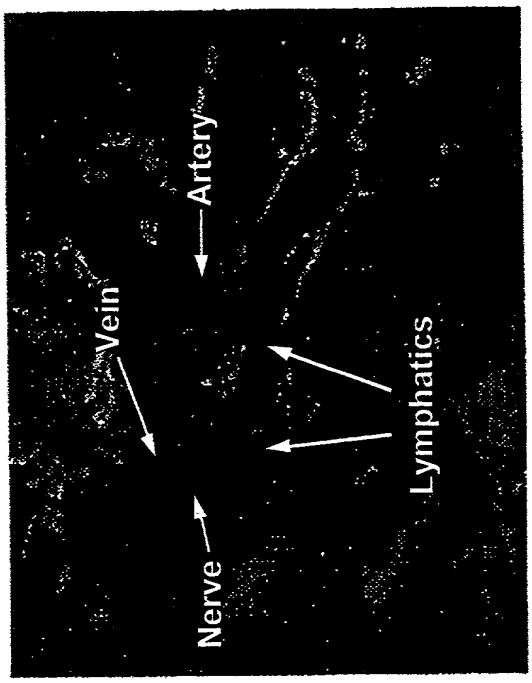


FIG. 12C

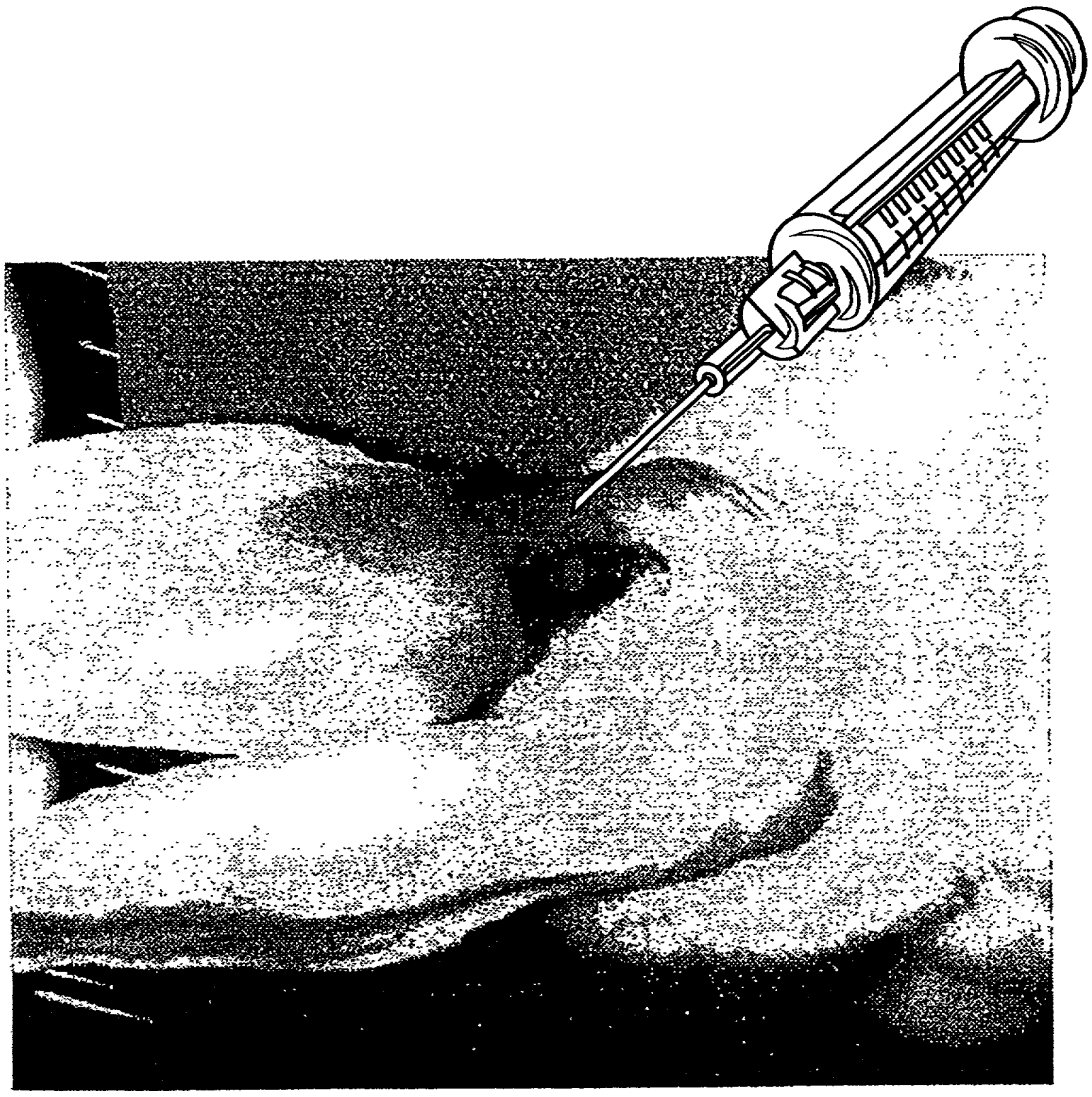


FIG. 13A

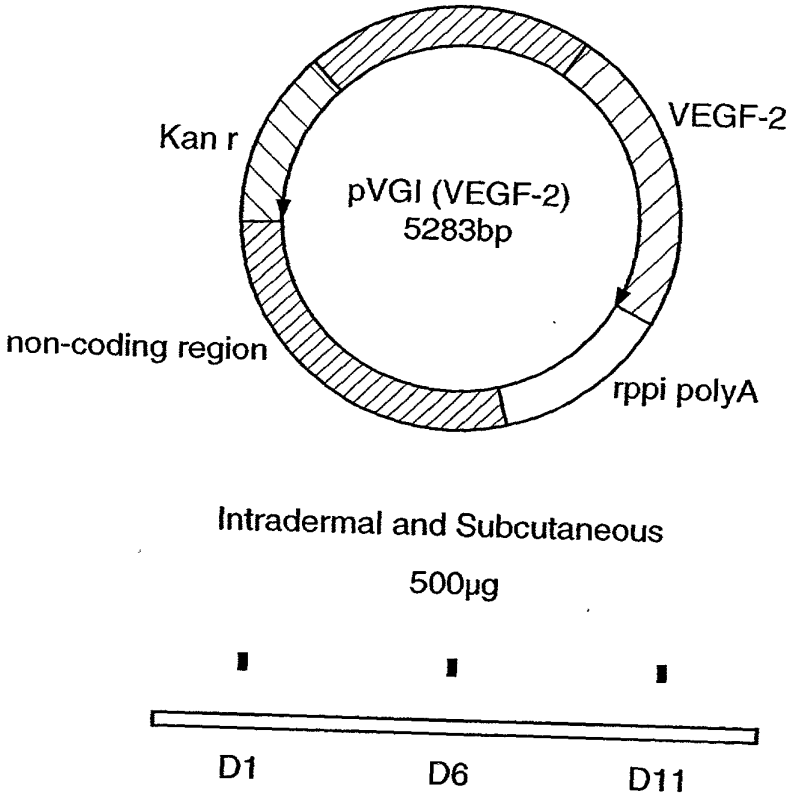

Kan r
VEGF-2
pVGI (VEGF-2)
5283bp
non-coding region
rppi polyA
Intradermal and Subcutaneous
500µg
D1
D6
D11

FIG. 13B

FIG. 14A

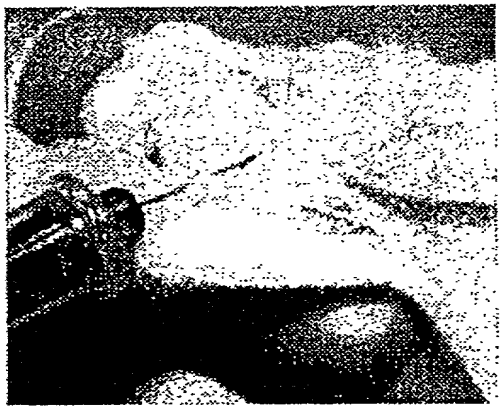


FIG. 14B

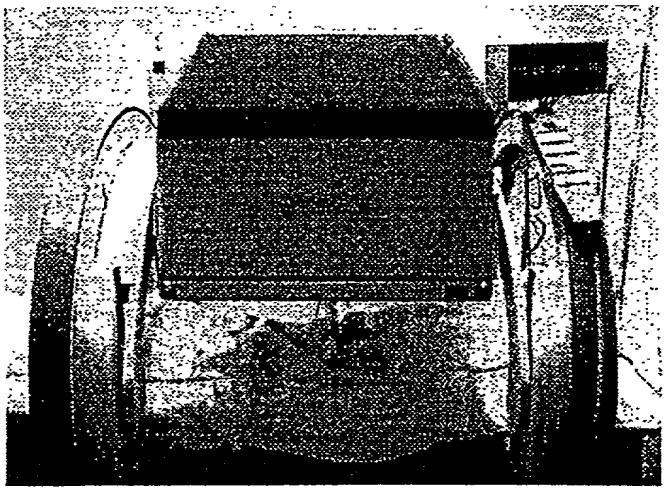
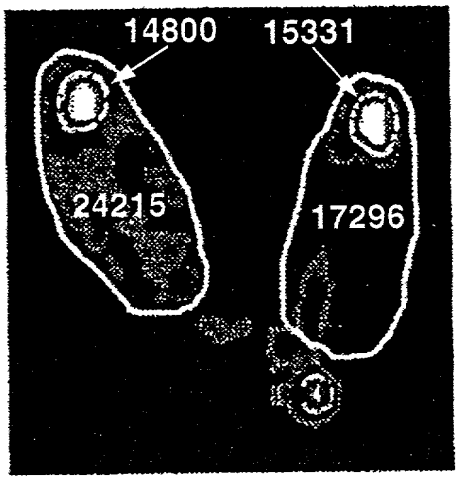
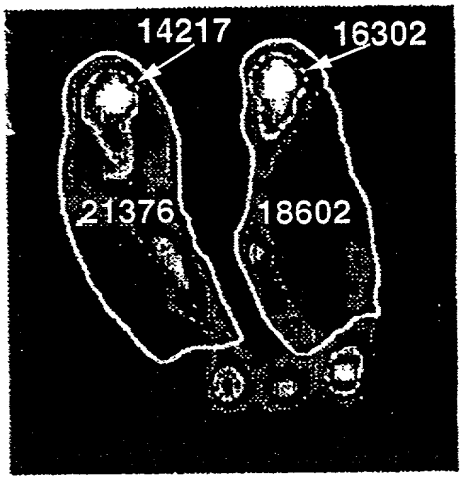


FIG. 14C



(24125-14800)/(17296-15331)
= 4.75

FIG. 15A



(21376-14217)/(18602-16302)
= 3.11

FIG. 15B

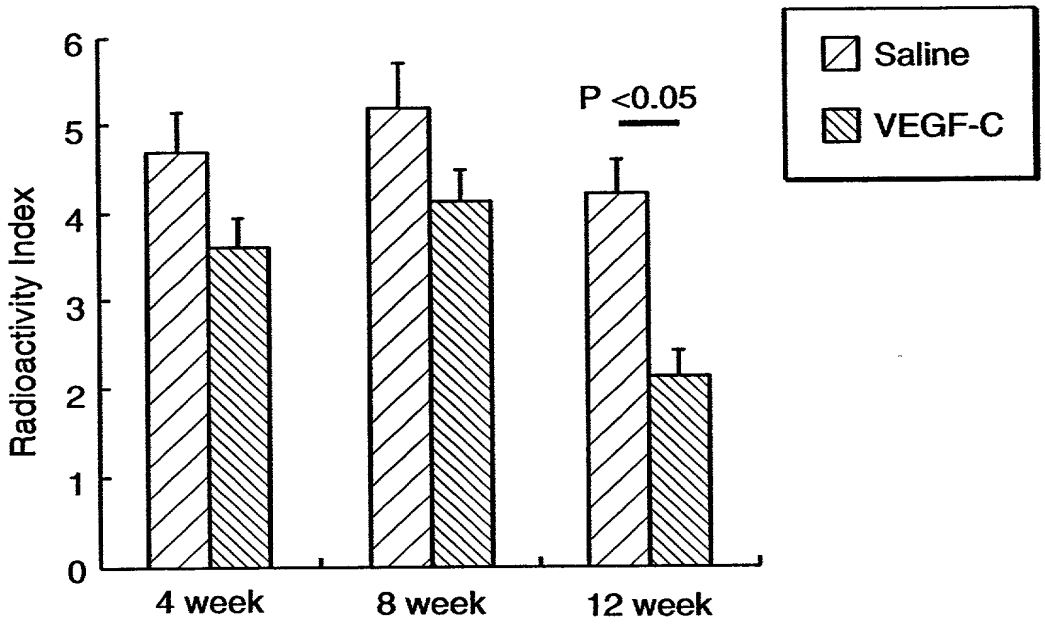


FIG. 15C

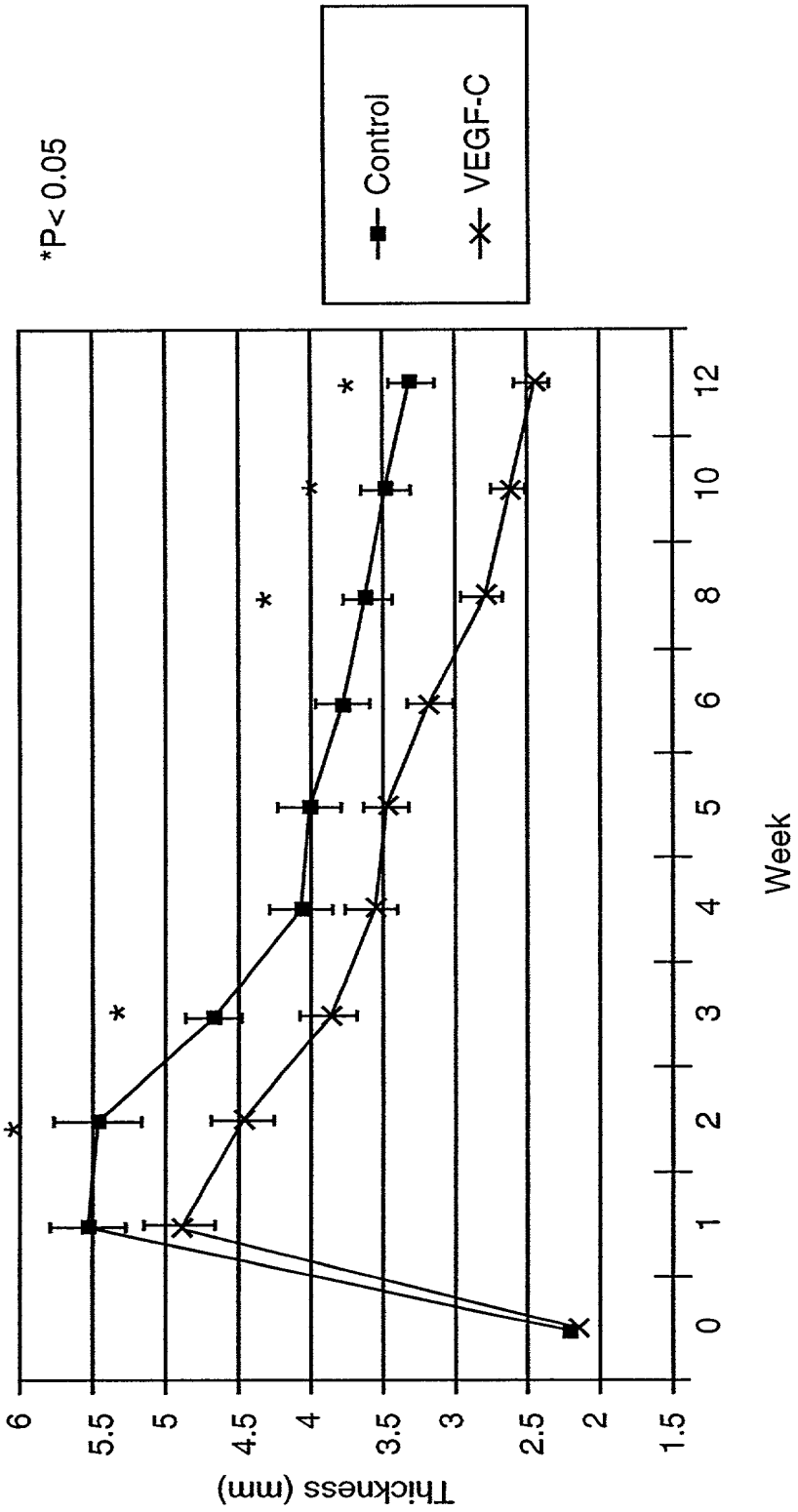

*P< 0.05
Control
VEGF-C
Thickness (mm)
Week
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
0
1
2
3
4
5
6
8
10
12

FIG. 16A

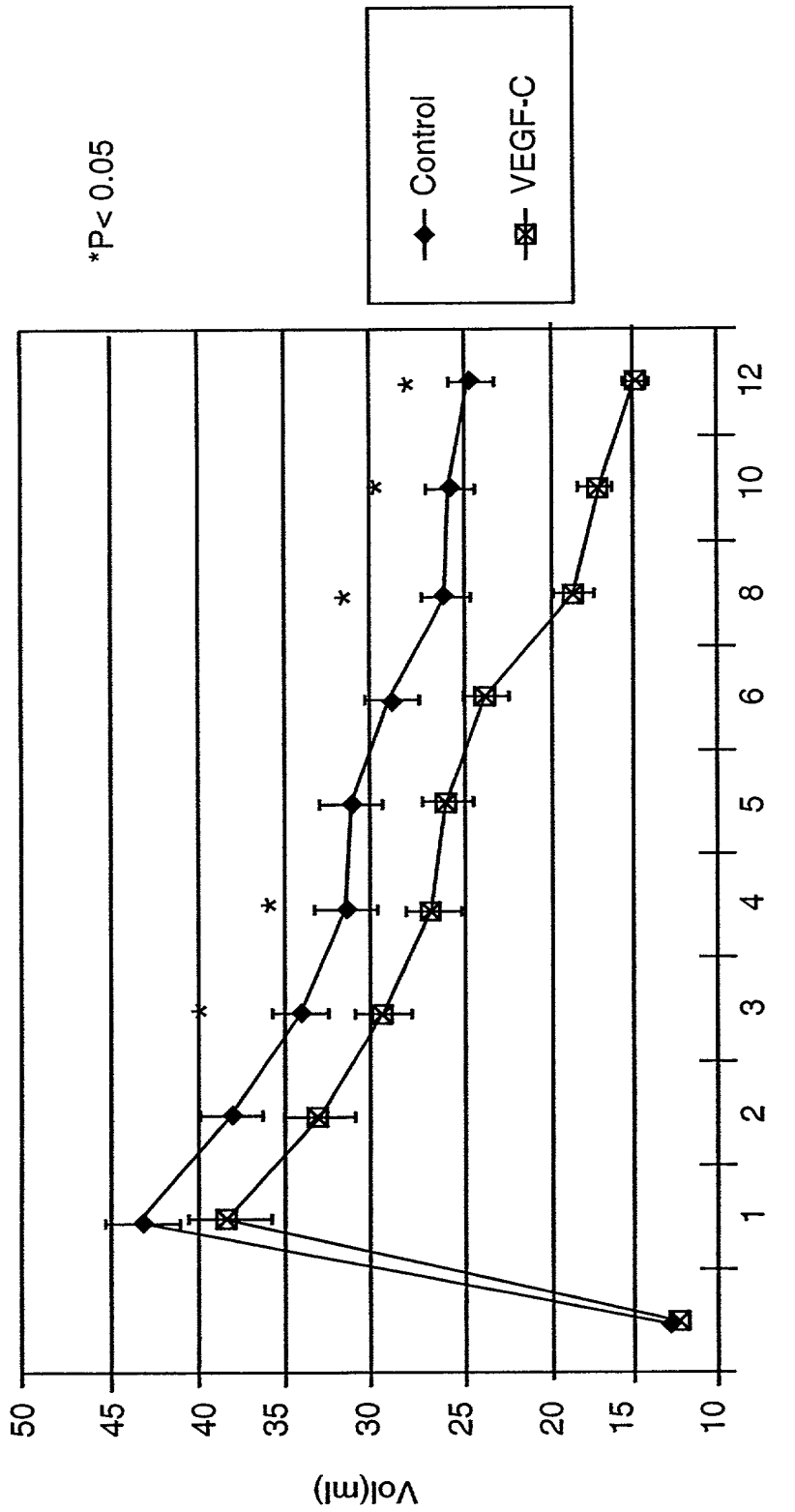
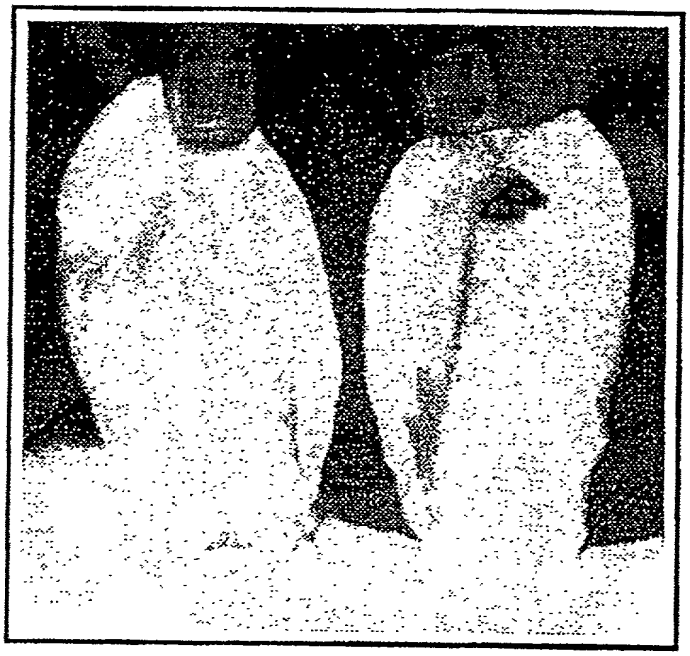

*P< 0.05
Control
VEGF-C
Vol(ml)
50
45
40
35
30
25
20
15
10
Week
1
2
3
4
5
6
8
10
12

FIG. 16B

FIG. 17A



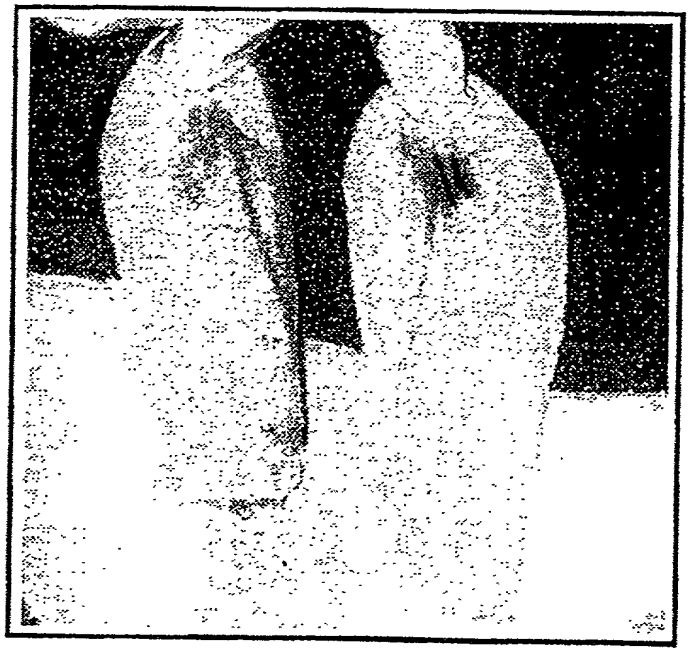


FIG. 17B

FIG. 17C

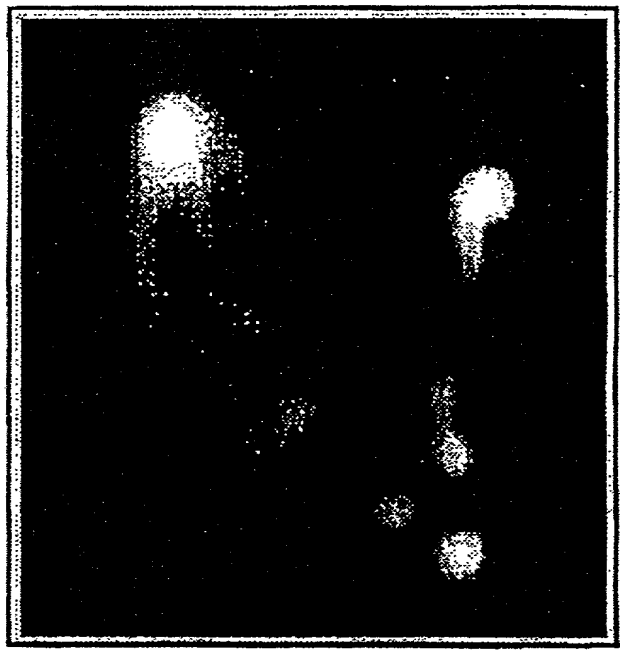


FIG. 17D

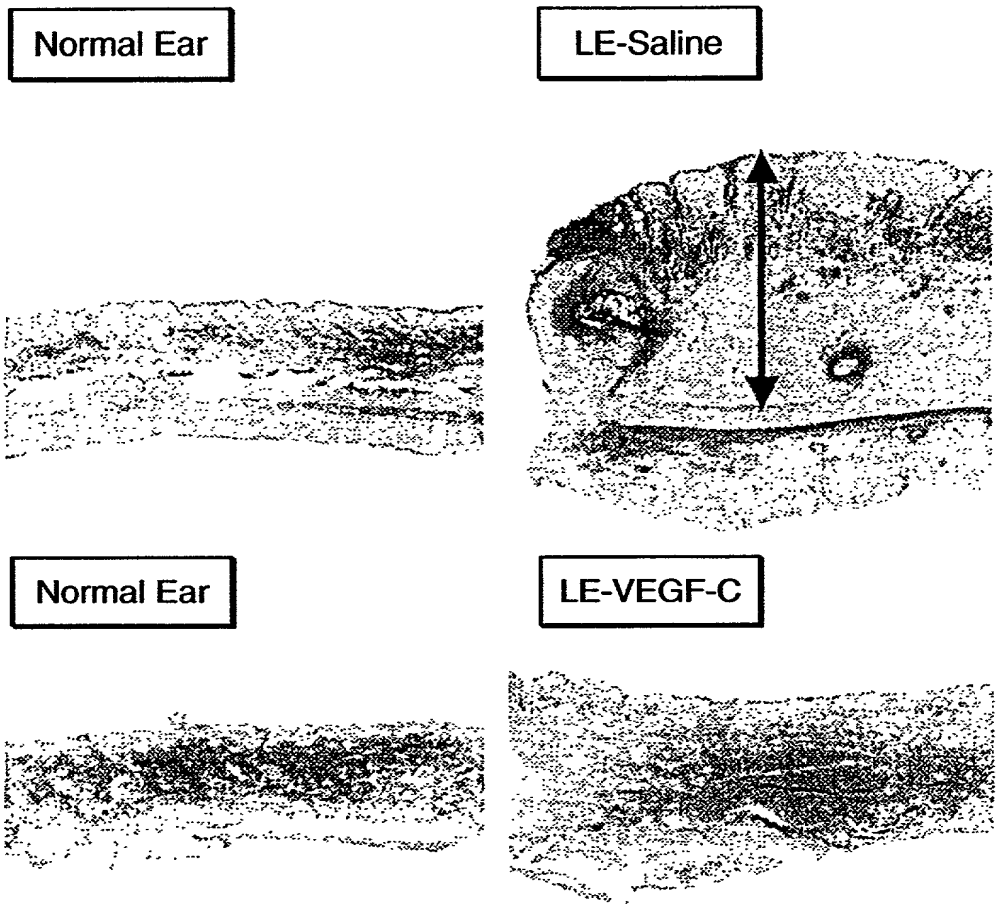


FIG. 18A

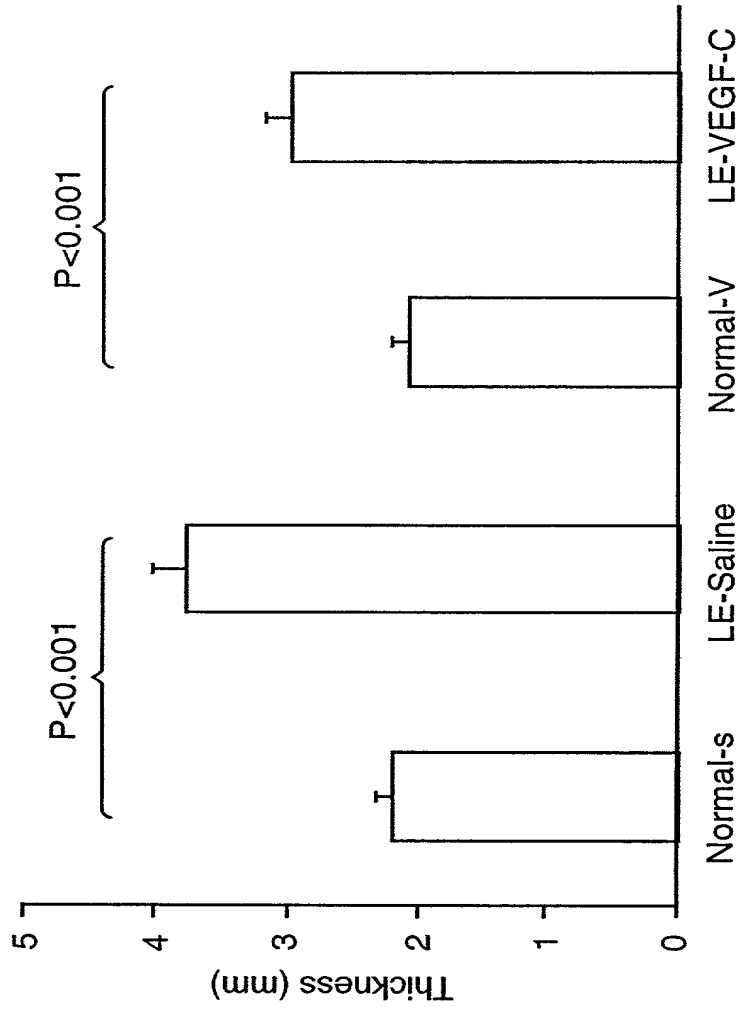

Thickness (mm)
0
1
2
3
4
5
Normal-s
LE-Saline
Normal-V
LE-VEGF-C
P<0.001
P<0.001
P<0.05

FIG. 18B

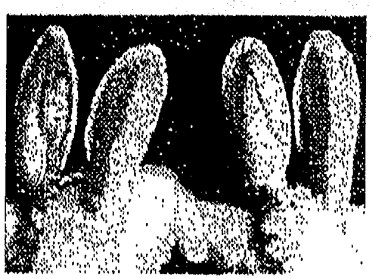


FIG. 19A

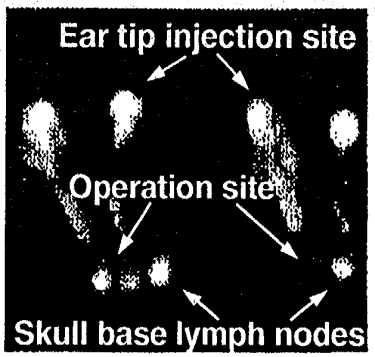


FIG. 19B

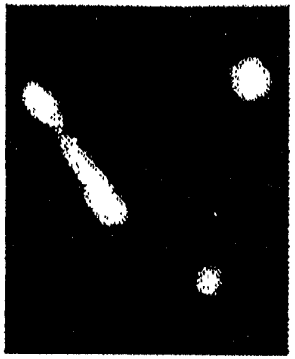


FIG. 19C

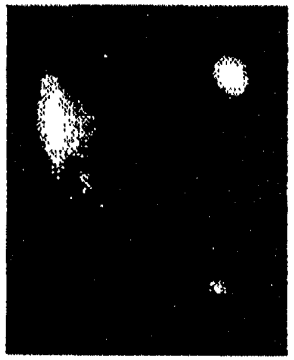


FIG. 19D

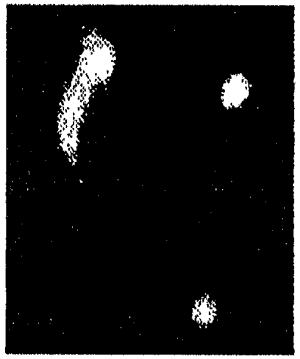


FIG. 19E

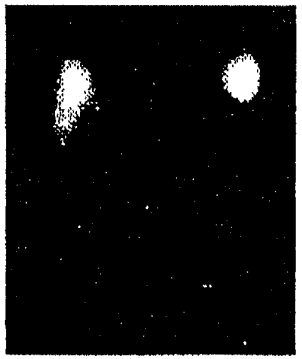


FIG. 19F

Title: Use of Lymphangiogenic Agents
To Treat Lymphatic Disorders
Inventor(s): Edwin C. Gravereaux, et al.
USSN 09/970,088

FIG. 19G

FIG. 19H

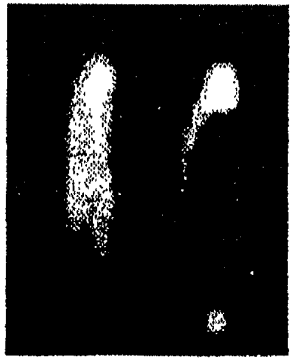


FIG. 19I

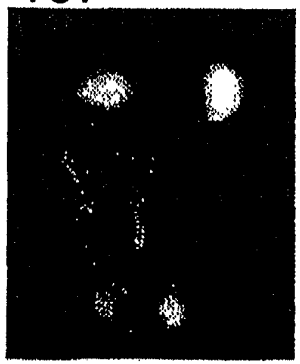


FIG. 19J

FIG. 20A

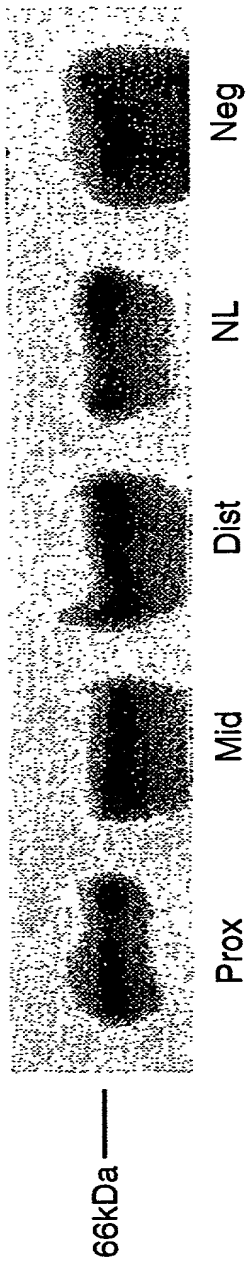


FIG. 20B

FIG. 21A
FIG. 21B

FIG. 21

```
rb    1    CGGTGCGCGG TGGCCGGGGC ACACGTGCCC AGCATCGTAT GGTACAAAGA
bo    1    CGGTGCCCAG TGGCTGGGAC GCACGTACCC AGCATCGTGT GGTACAAAGA
hu    1    CAGTGCTTGG TGGCCGGAGC GCACGCGCCC AGCATCGTGT GGTACAAAGA
mo    1    CGATGCCCGG TGGCTGGAGC GCATGTGCCC AGTATTGTGT GGTACAAAGA

rb   51    TGAGAGGCTG CTGCAAGAAG AATCTGGAAT CGACCTCGCG GACTCGAACC
bo   51    TGAGAAGCTG CTGGAAGAAG AGTCCGGAAT CGACCTGGCG GACTCGAACC
hu   51    CGAGAGGCTG CTGGAGGAAA AGTCTGGAGT CGACTTGGCG GACTCCAACC
mo   51    TGAAAGGCTC CTGGAGAAAG AGTCGGGAAT CGACCTGGCA GACTCCAATC

rb  101    AGAGGCTGAG CATCCAGCGC GTGCGCGAGG AGGACGCGGG CCGCTATCTG
bo  101    AGAGGCTGAG CATCCAGCGC GTGCGCGAGG AGGACGCGGG CCACTATCTG
hu  101    AGAAGCTGAG CATCCAGCGC GTGCGCGAGG AGGATGCGGG ACGCTATCTG
mo  101    AGAGGCTGAG CATCCAGCGC GTGCGCGAGG AGGACGCAGG TCGTTATCTG

rb  151    TGCAGCGTGT GCAACGCCAA GGGCTGCGTC AACTCCTCCG CCAGCGTAGC
bo  151    TGCAGTGTGT GCAACGCCAA GGGCTGTGTC AACTCCTCTG CCAGCGTGGC
hu  151    TGCAGCGTGT GCAACGCCAA GGGCTGCGTC AACTCCTCCG CCAGCGTGGC
mo  151    TGCAGCGTGT GCAATGCCAA GGGCTGCGTA AACTCCTCTG CCAGCGTGGC

rb  201    TGTGGGAGGC GCCGAAGATA GAGGCAGCAT GGAGATCGTG ATCCTCGTGG
bo  201    TGTGGAAGGC TCTGAGGATA AAGGCAGCAT GGAGATCGTG ATCCTTGTTG
hu  201    CGTGGAAGGC TCCGAGGATA AGGGCAGCAT GGAGATCGTG ATCCTTGTCG
mo  201    AGTGGAAGGC TCTGAAGATA AAGGCAGCAT GGAGATTGTG ATACTCATTG
```

FIG. 21A

rb	251	GCACCGGCGT	CATTGCCGTG	TTCTTTTGGG	TCCTCCTCCT	GCTCATCTTC
bo	251	GCACCGGAGT	CATCGCTGTC	TTTTTCTGGG	TCCTCCTTCT	CCTCATCTTC
hu	251	GTACCGGCGT	CATCGCTGTC	TTCTTCTGGG	TCCTCCTCCT	GCTCATCTTC
mo	251	GCACCGGCGT	CATCGCAGTT	TTCTTCTGGG	TCCTCCTCCT	GCTCATCTTC
rb	301	TGTAACATGA	GGAGGCCAGC	CCACGCGGAC	ATCAAGACGG	GCTACTTGTC
bo	301	TGTAACATGA	GGAGGCCAAC	CCATGCAGAC	ATCAAGACTG	GCTACTTGTC
hu	301	TGTAACATGA	GGAGGCCGGC	CCACGCAGAC	ATCAAGACGG	GCTACCTGTC
mo	301	TGTAACATGA	AAAGGCCTGC	CCATGCAGAC	ATCAAGACGG	GCTACCTGTC
rb	351	CATCATCATG	GATCCCGGGG	AGGTGCCTCT	GGAGGAGCAA	TGTGAATACC
bo	351	CATCATCATG	GACCCCGGGG	AGGTGCCTTT	GGAGGAGCAG	TGTGAATACC
hu	351	CATCATCATG	GACCCCGGGG	AGGTGCCTCT	GGAGGAGCAA	TGCGAATACC
mo	351	CATCATCATG	GACCCCGGGG	AGGTGCCTTT	GGAGGAGCAG	TGTGAATACC
rb	401	TGTCCTACGA	CGCCAGCCAG			
bo	401	TGTCCTACGA	TGCTAGTCAA			
hu	401	TGTCCTACGA	TGCCAGCCAG			
mo	401	TGTCCTATGA	CGCCAGCCAG			

FIG. 21B

```
rb      1  RCAVAGAHVP SIVWVKDERL LEEESGIDLA DSNQRLSIQR VREEDAGRVL
bo      1  RCPVAGTHVP SIVWVKDEKL LEEESGIDLA DSNQRLSIQR VREEDAGHVL
hu      1  QCLVAGAHAP SIVWVKDERL LEEKSGVDLA DSNQKLSIQR VREEDAGRVL
mo      1  RCPVAGAHVP SIVWVKDERL LEKESGIDLA DSNQRLSIQR VREEDAGRVL

rb     51  CSVCNAKGCV NSSASVAVGG AEDRGSMEIV ILVGTGVIAV FFWVLLLLIF
bo     51  CSVCNAKGCV NSSASVAVEG SEDKGSMEIV ILVGTGVIAV FFWVLLLLIF
hu     51  CSVCNAKGCV NSSASVAVEG SEDKGSMEIV ILVGTGVIAV FFWVLLLLIF
mo     51  CSVCNAKGCV NSSASVAVEG SEDKGSMEIV ILIGTGVIAV FFWVLLLLIF

rb    101  CNMRRPAHAD IKTGVLSIIM DPGEVPLEEQ CEVLSYDASQ
bo    101  CNMRRPTHAD IKTGVLSIIM DPGEVPLEEQ CEVLSYDASQ
hu    101  CNMRRPAHAD IKTGVLSIIM DPGEVPLEEQ CEVLSYDASQ
mo    101  CNMRRPAHAD IKTGVLSIIM DPGEVPLEEQ CEVLSYDASQ
```

FIG. 22A

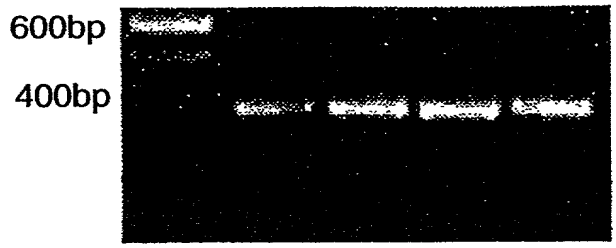


FIG. 22B

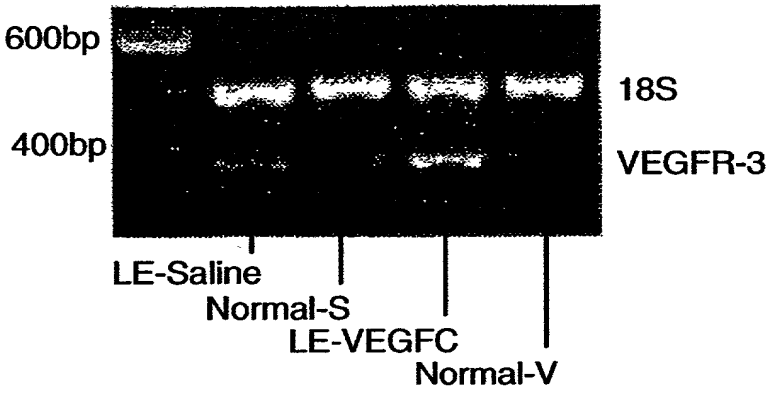


FIG. 22C

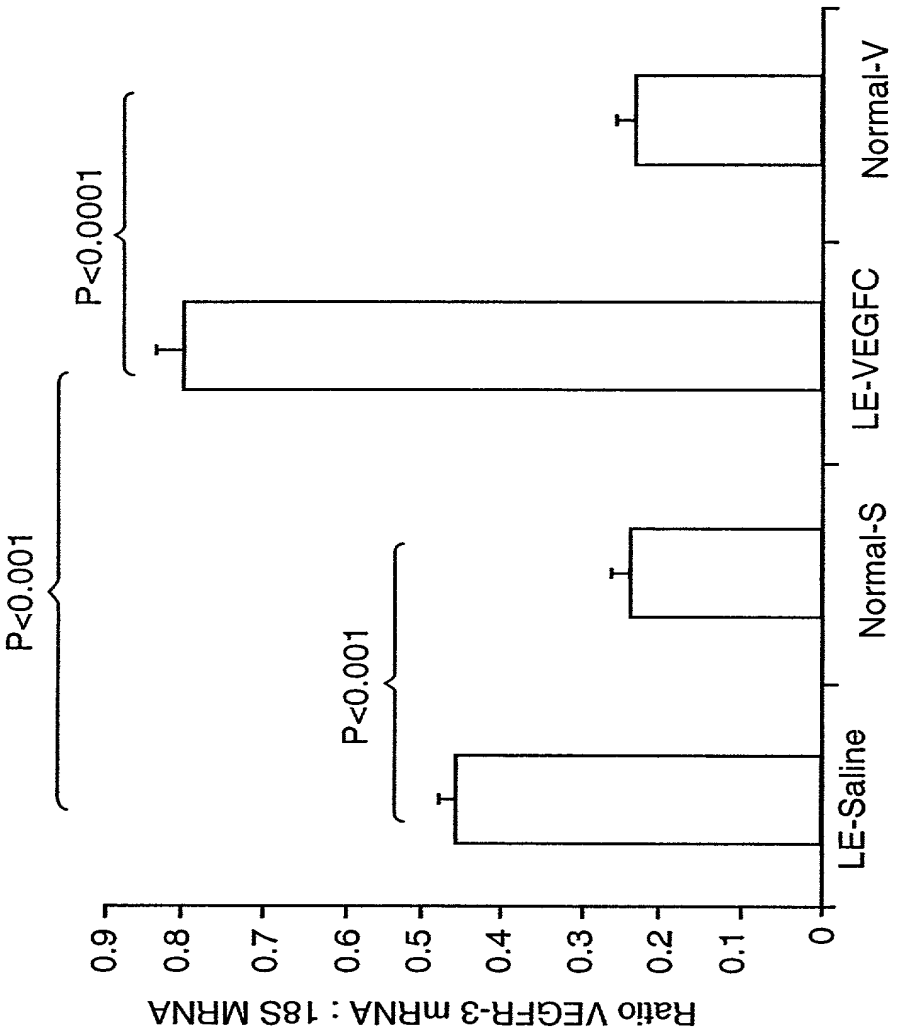

Ratio VEGFR-3 mRNA : 18S MRNA
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
P<0.001
P<0.001
P<0.0001
LE-Saline
Normal-S
LE-VEGFC
Normal-V

FIG. 22D

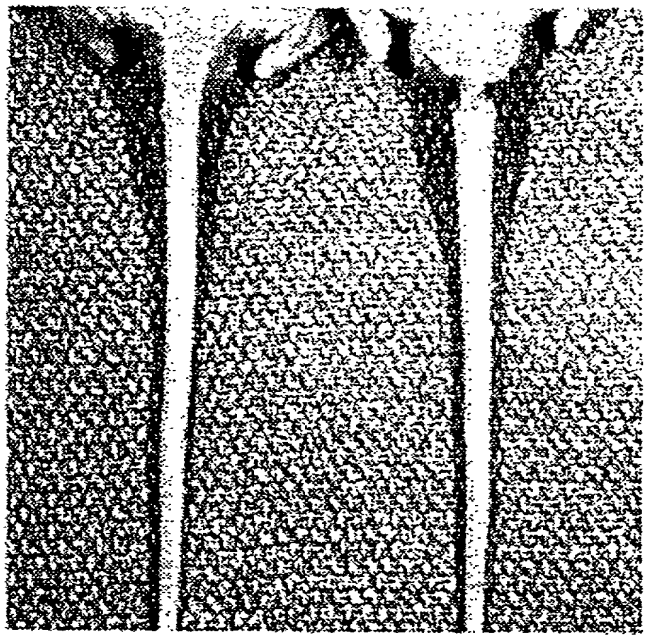


FIG. 23A

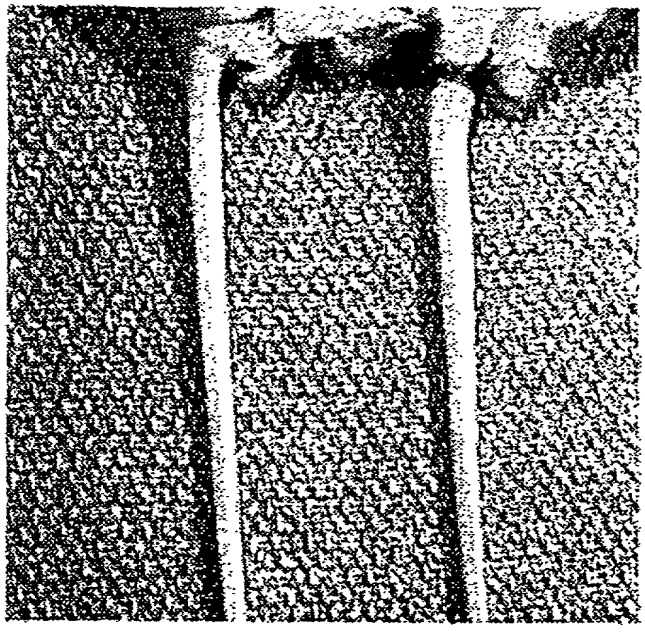


FIG. 23B

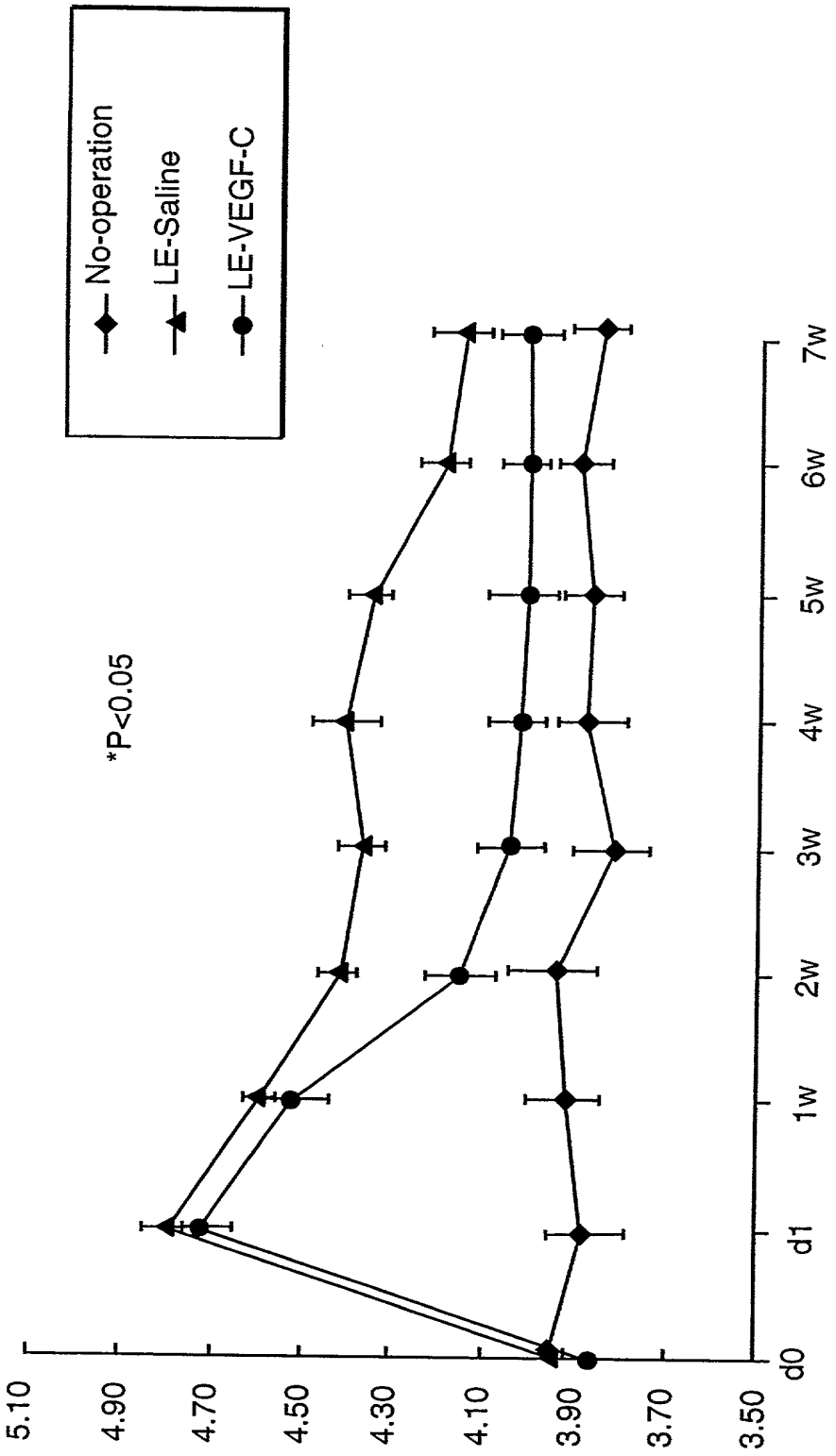

No-operation
LE-Saline
LE-VEGF-C
*P<0.05
5.10
4.90
4.70
4.50
4.30
4.10
3.90
3.70
3.50
d0
d1
1w
2w
3w
4w
5w
6w
7w

FIG. 23C

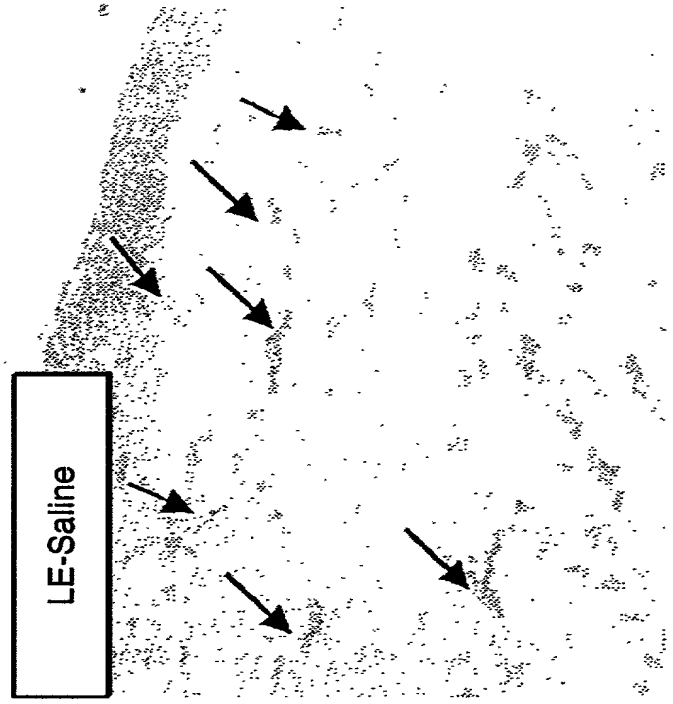


FIG. 24B

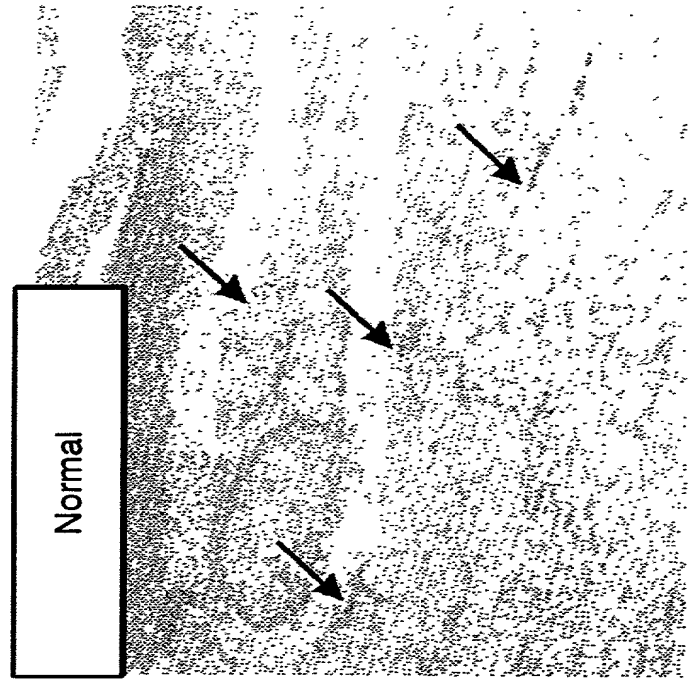


FIG. 24A

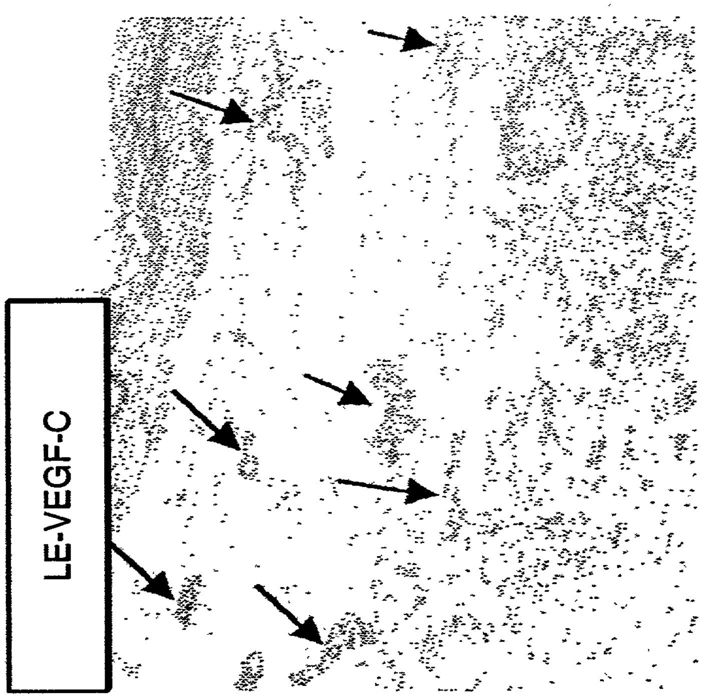


FIG. 24C

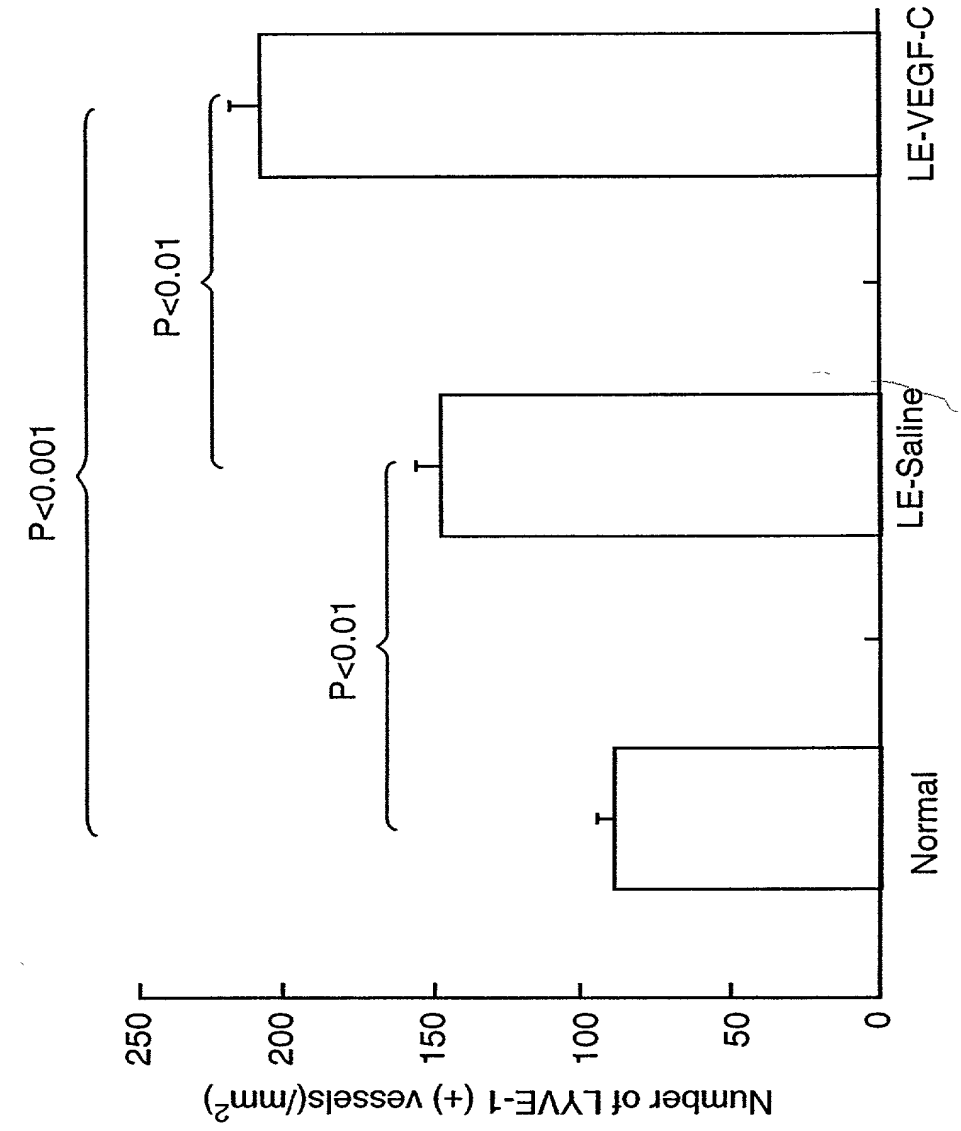

Number of LYVE-1 (+) vessels(/mm²)
250
200
150
100
50
0
Normal
LE-Saline
LE-VEGF-C
P<0.001
P<0.01
P<0.01

FIG. 24D